**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**
**WASHINGTON, D.C. 20460**

**OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION**

**MEMORANDUM**

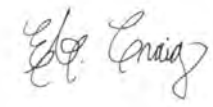
May 29, 2019

**SUBJECT:** **Tribufos:** Review of *In Vitro* Dermal Absorption study in humans, rats, and rabbits

**PC Code:** 074801
**Decision No.:** 545589
**Petition No.:** N/A
**Risk Assessment Type:** N/A
**TXR No.:** 0057866
**MRID No.:** 50563601

**DP Barcode:** 449544
**Registration No.:** N/A
**Regulatory Action:** Registration review
**Case No.:** N/A
**CAS No.:** 78-48-8
**40 CFR:** N/A

**FROM:** Evisabel Craig PhD, DABT, Toxicologist
Risk Assessment Branch VI
Health Effects Division (7509P)



**THROUGH:** Richard Fehir PhD, Acting Branch Chief
Risk Assessment Branch VI
Health Effects Division (HED) (7509P)

**TO:** Kelly Sherman, Branch Chief
Marianne Mannix, Chemical Review Manager
Tracy Perry, Team Leader
Risk Management and Implementation Branch III
Pesticide Re-Evaluation Division (PRD) (7508P)

**I. ACTION REQUESTED:** PRD requested that HED review a recently submitted *in vitro* dermal absorption study for tribufos.

**II. RESULTS:** The submitted dermal absorption study (MRID 50563601) was reviewed and a data evaluation report was created.

**III. CONCLUSIONS:** The percentage of the applied dose that was absorbed was lowest for human skin, increased for rat skin, and greatest for rabbit skin. The mid and low doses correspond to in-use application rates of the product. The *in vitro* dermal absorption for tribufos is ~8% in humans, ~37% in rats and ~46% in rabbits.

This study is classified **acceptable / non-guideline.**

# DATA EVALUATION RECORD

TRIBUFOS

Study Type: OCSPP Non-Guideline; *In Vitro* Dermal Absorption Study
(Human, Rat, and Rabbit Skin)

Work Assignment No. 32-3-018 (MRID 50563601)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by

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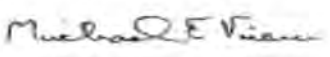
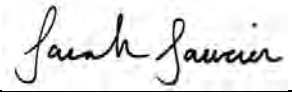
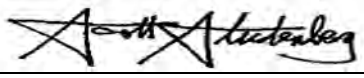
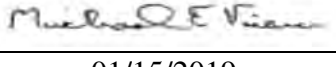
| | | |
|---|---|---|
| Primary Reviewer:<br>Michael E. Viana, Ph.D., DABT | Signature:<br>Date: |  <br>01/02/2019 |
| Secondary Reviewer:<br>Sarah E. Saucier, Ph.D. | Signature:<br>Date: |  <br>01/12/2019 |
| Quality Assurance:<br>Scott D. Studenberg, Ph.D., DABT | Signature:<br>Date: |  <br>01/15/2019 |
| Project Manager:<br>Michael E. Viana, Ph.D., DABT | Signature:<br>Date: |  <br>01/15/2019 |

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. Contractor's role did not include establishing Agency policy.

**EPA Reviewer:** Evisabel Craig **Signature:** Evi Craig

**Risk Assessment Branch VI, Health Effects Division (7509P)** **Date:** 5/29/2019

Template version 02/06

## DATA EVALUATION RECORD

**STUDY TYPE:** *In Vitro* Dermal Absorption Study (Human, Rat, and Rabbit Skin), OCSPP Non-Guideline; OECD 428.

**PC CODE:** 074801 **DP BARCODE:** 449544
**TXR #:** 0057866

**TEST MATERIAL (RADIOCHEMICAL PURITY):** [$^{14}$C]-tribufos (99.9%)

**SYNONYMS:** *S,S,S*-tributyl phosphorotrithioate

**CITATION:** Jones, A. (2018) Tribufos: Comparative *In Vitro* Dermal Absorption using Human, Rat and Rabbit Skin. Envigo CRS Ltd., Alconbury, Huntingdon, Cambridgeshire, UK. Laboratory Study No.: LG35SC, March 28, 2018. MRID 50563601. Unpublished.

**SPONSOR:** AMVAC Chemical Corporation USA, Glen A. Wintemute Research Center, 2110 Davie Avenue, Commerce, CA.

**EXECUTIVE SUMMARY:** In an *in vitro* dermal absorption study (MRID 50563601), [$^{14}$C]-tribufos (99.9% radiochemical purity; Lot #: 372-085-0586-A-20170313-PVA) was applied to human, rat, and rabbit epidermal membranes in flow-through diffusion cells. Membranes were exposed to tribufos for a period of 10 hours, after which they were washed with a mild detergent. Half the membranes were terminated at 10 hours, while the other half were allowed to continue until 24 hours post-application. Receptor fluid samples were collected every two hours through 24 hours post-application. All skin membranes were swabbed at 10-h post-application. The target doses for the dilutions were 7200, 101, or 10.1 µg/cm$^2$ (4608, 64.64, or 6.464 µg equiv., respectively).

Acceptable total recoveries of ≥90% were obtained from all skin sections.

The percentage of radioactive residues (relative to the applied dose) that were removed from the site of application by the 10-h skin wash was greatest for human skin samples, followed by rat skin, then by rabbit skin. Residues removed from human skin were 88-93% of the applied dose for the high- and low-dose groups, with 60-66% of the applied dose removed from the intermediate-dose group. Residues removed from rat skin were 36-57% of the applied dose for all dose groups; residues removed from rabbit skin were 17-41% of the applied dose for all dose groups. For the 24-h exposure experiments, additional residues removed from human skin samples were 2.1% of the applied dose for both the high- and low-dose groups with values of

13.3% for the intermediate-dose group. Residues removed at 24 h from rat skin samples were 4-12% of the applied dose for all dose groups; residues removed from rabbit skin were 1-8% for all dose groups.

For the human skin membranes, 50% of total absorption had occurred by 8 h or 12 h in the high-dose groups, by 4 h or 16 h in the intermediate-dose groups, and by 6 h and 14 h in the low-dose groups after the end of the 10-h and 24-h periods, respectively. For the rat skin membranes, 50% of total absorption had occurred by 6 h or 8 h in the high-dose groups, by 8 h or 14 h in the intermediate-dose groups, and by 8 h and 16 h in the low-dose groups after the end of the 10-h and 24-h periods, respectively. For the rabbit skin membranes, 50% of total absorption had occurred by 4 h or 12 h in the high-dose groups, by 8 h or 16 h in the intermediate-dose groups, and by 8 h and 12 h in the low-dose groups after the end of the 10-h and 24-h periods, respectively. These data demonstrate that absorption of [$^{14}$C]-tribufos through human, rat, and rabbit skin continued after the 10-h skin wash. These data also provide support that radioactive residues of tribufos that remained in the stratum corneum should be considered available for absorption.

Total potentially-absorbable tribufos was reported as the sum of radioactive residues present in the receptor fluid, the skin, the stratum corneum (tape strips 3-last), and residual residues from the receptor chamber. Radioactive materials removed by washing at 10 h or 24 h, recovered from the surface (tape strips 1-2) at the end of the exposure period, and any residual material in the donor chamber were considered unabsorbed. The percentage of the applied dose that was absorbed was lowest for human skin, increased for rat skin, and greatest for rabbit skin. The mid and low doses correspond to in-use application rates of the product. For rabbit skin, absorption was similar across doses and time points (44-48%). For human skin, higher absorption was observed at the mid dose. In addition, the low dose group for human skin had several samples that were excluded because they did not meet the acceptance criteria for membrane integrity. Therefore, the % dermal absorption was selected from the mid dose dermal absorption rates. There were no major differences in absorption in samples collected at the 10 and 24-hour timepoints; therefore the *in vitro* dermal absorption for tribufos is ~8% in humans, ~37% in rats and ~46% in rabbits.

This study is classified **acceptable / non-guideline.** It was stated that it was performed in accordance in compliance with:

- Section 7.3 of Annex – Part A of Commission Regulation (EU) No. 545/2011 using the OECD Test Guideline 428 (April 2004) and the corresponding OECD Guidance Document for conducting *in vitro* skin absorption studies (March 2004); and
- Guidance on Dermal Absorption, EFSA Journal 2012; 10(4):2665

**COMPLIANCE:** Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided. It was stated that the present study was conducted in compliance with:

- UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994);

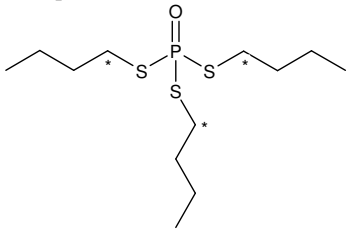
- OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17; and
- EC Commission Directive 2004/10/EC of February 11, 2004 (Official Journal No. L 50/44).

# I. MATERIALS AND METHODS

## A. MATERIALS

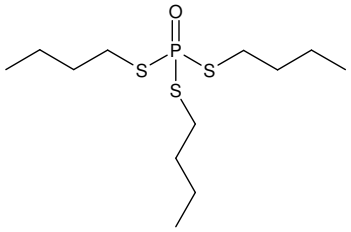
### 1. Test material

**Radiolabeled test material:** [$^{14}$C]-Tribufos
**Radiochemical purity:** ≥99% (HPLC)
**Specific activity:** 6.85 MBq/mg (58.6 mCi/mol)
**Lot No.:** 372-085-0586-A-20170313-PVA
**Expiration / Storage:** Not reported / −20°C ± 10°C
**Structure:**



* denotes position of radiolabel

**Non-radiolabeled TGAI:** Tribufos
**Description:** Liquid
**Batch #:** 200-19-1
**Purity:** 96.2%
**CAS # of TGAI:** 78-48-8
**Expiration / Storage:** May 3, 2019 / refrigerated and desiccated under nitrogen
**Structure:**



**Formulated product:** Folex 6EC
**Description:** Amber liquid
**Batch #:** SS16250
**Purity:** 72%
**Expiration / Storage:** February 12, 2020 / refrigerated under nitrogen

**Formulation blank:** Folex 6EC blank
**Batch #:** SS17101
**Expiration / storage** April 11, 2019 / refrigerated under nitrogen

**2. Relevance of test material to proposed formulation(s):** Tribufos is a commercially available organothiophosphate used as a defoliant for cotton plants. The proposed formulations represent mixtures of [$^{14}$C]-labeled tribufos combined with non-labeled TGAI, the commercial emulsifiable concentrate, and/or the formulation blank to yield the desired concentrations. These concentrations represent potential exposure levels for loaders and applicators in field work.

## 3. Test systems

| | | |
|---|---|---|
| **Species:** | Human skin (females only) | |
| **Source:** | Whole skin samples obtained during elective surgery from Tissue Solutions (address not provided) | |
| **Age:** | 35-66 yrs | |
| **Origin/Anatomical site:** | Abdomen | |
| | | |
| **Species:** | Rat (males only) | |
| **Strain:** | Sprague-Dawley [Crl:CD(SD)] | |
| **Age / mean weight at euthanasia:** | 30 days / 97-135 g | |
| **Source:** | Charles River UK Ltd. (Margate, Kent, UK) | |
| **Housing:** | Up to five rats/cage in polycarbonate cages with solid floors | |
| **Diet:** | VRF1 (Special Diet Services, Witham, UK), *ad libitum* | |
| **Water:** | Tap water, *ad libitum* | |
| **Environmental conditions:** | **Temperature:** | 22 ± 2°C |
| | **Humidity:** | 55 ± 15% |
| | **Air changes:** | Not reported |
| | **Photoperiod:** | 12 h light/12 h dark |
| **Acclimation period:** | At least five days | |
| | | |
| **Species:** | Rabbit (males only) | |
| **Strain:** | New Zealand White | |
| **Age / mean weight at euthanasia:** | 77 days / 2.14-2.38 kg | |
| **Source:** | Envigo RMS UK Ltd. | |
| **Housing:** | Individually in suspended plastic cages with perforated floor panels | |
| **Diet:** | Teklad 2930C (supplier not provided) | |
| **Water:** | Tap water, *ad libitum* | |
| **Environmental conditions:** | **Temperature:** | 18 ± 3°C |
| | **Humidity:** | 55 ± 15% |
| | **Air changes:** | Not reported |
| | **Photoperiod:** | 12 h light/12 h dark |
| **Acclimation period:** | At least five days | |

# B. TEST SYSTEM AND STUDY DESIGN

## 1. Dose

**Dose selection rationale:** Three concentrations were tested corresponding to the commercially available emusifiable concentrate (720 g/L), and two in-use application rates of the test material (10.1 g/L and 1.01 g/L). It was also stated that the two lower concentrations were previously tested in a rat dermal absorption study (MRID 42350003; Schroeder 1993, not provided). Theoretical and actual doses achieved are shown in Table 1.

**High-dose preparation:** A 1.13 mg aliquot of [$^{14}$C]-tribufos stock solution was evaporated with nitrogen, combined with 2 mL of the emusifiable concentrate, and stirred.

**Intermediate-dose preparation:** A 1.09 mg aliquot of [$^{14}$C]-tribufos was evaporated with nitrogen, combined with 19.22 mg of non-labeled tribufos and 7.94 mg of formulation blank, and adjusted to a final volume of 2 mL with water. The formulation was vortexed and stirred.

**Low-dose preparation:** A 2.06 mg aliquot of [$^{14}$C]-tribufos was evaporated with nitrogen, combined with 0.78 mg of formulation blank, and adjusted to a final volume of 2 mL with water. The formulation was stirred.

**Nominal doses:** 7200 µg/cm$^2$ (720 g/L), 101 µg/cm$^2$ (10.1 g/L) and 10.1 µg/cm$^2$ (1.01 g/L), respectively.

**Homogeneity (% CV; calculated by the Reviewers):** 0.3-4.7%
**Stability:** Not reported

**Dose volume:** 10 µL/cm$^2$

**Termination periods:** 10 or 24 h

**Number of skin preparations/dose group/dose duration**
Human: ten
Rat and rabbit: six

**TABLE 1.** Dosing [a]

| Group | Termination time (h) | Theoretical | | | Actual | | |
|---|---|---|---|---|---|---|---|
| | | Level | Dose a.i. (µg/cm$^2$) | Amount a.i. (µg equiv) | Dose a.i. (µg/cm$^2$) [b] | Amount a.i. (µg equiv) [b] | Specific activity (dpm/µg) [b] |
| **Human** | | | | | | | |
| 1 | 10 | High | 7200 | 4608 | 7003 ± 180 | 4482 ± 115 | 326.01 ± 0.02 |
| | 24 | | 7200 | 4608 | 7037 ± 191 | 4504 ± 122 | 325.99 ± 0.02 |
| 2 | 10 | Intermediate | 101 | 64.64 | 106.6 ± 0.0 | 68.2 ± 0.0 | 21,134 ± 0.0 |
| | 24 | | 101 | 64.64 | 99.8 ± 2.7 | 63.9 ± 1.8 | 22,121 ± 12.3 |
| 3 | 10 | Low | 10.1 | 6.464 | 9.5 ± 0.6 | 6.1 ± 0.4 | 411,218 ± 1773 |
| | 24 | | 10.1 | 6.464 | 9.6 ± 0.3 | 6.1 ± 0.2 | 411,550 ± 3371 |
| **Rat** | | | | | | | |
| 4 | 10 | High | 7200 | 4608 | 7043 ± 114 | 4508 ± 73 | 326.01 ± 0.01 |
| | 24 | | 7200 | 4608 | 7086 ± 74 | 4535 ± 47 | 326.01 ± 0.02 |
| 5 | 10 | Intermediate | 101 | 64.64 | 101.6 ± 2.4 | 65.0 ± 1.5 | 22,128 ± 9.4 |
| | 24 | | 101 | 64.64 | 100.6 ± 4.0 | 64.4 ± 2.6 | 22,116 ± 8.0 |
| 6 | 10 | Low | 10.1 | 6.464 | 9.1 ± 0.4 | 5.8 ± 0.2 | 410,766 ± 2391 |
| | 24 | | 10.1 | 6.464 | 9.3 ± 0.4 | 5.9 ± 0.3 | 411,666 ± 1626 |
| **Rabbit** | | | | | | | |
| 7 | 10 | High | 7200 | 4608 | 6770 ± 336 | 4333 ± 215 | 326.00 ± 0.01 |
| | 24 | | 7200 | 4608 | 7017 ± 99 | 4491 ± 63 | 325.99 ± 0.02 |
| 8 | 10 | Intermediate | 101 | 64.64 | 102.3 ± 1.9 | 65.5 ± 1.2 | 22,120 ± 8.8 |
| | 24 | | 101 | 64.64 | 103.1 ± 1.2 | 66.0 ± 0.8 | 22,122 ± 11.6 |
| 9 | 10 | Low | 10.1 | 6.464 | 9.2 ± 0.3 | 5.9 ± 0.2 | 409,543 ± 825 |
| | 24 | | 10.1 | 6.464 | 9.4 ± 0.3 | 6.0 ± 0.2 | 411,816 ± 2354 |

a Data were obtained on pages 20, 27, and 106-111 (Appendix 3) of MRID 50563601.
b Mean (±SD) calculated by the Reviewers from individual data.

**2. <u>Receptor fluid</u>:** The solubility of tribufos in several potential receptor fluids was investigated. The investigation was conducted by determining if an equivalent amount of tribufos as applied at the high-dose (approximately 4608 µg in 6.4 µL) was soluble in the total volume of receptor fluid collected over the duration of the experiment (36 mL) to ensure solubility was not a rate-limiting factor. The receptor fluids examined were 5%

(w/v) or 10% (w/v) bovine serum albumin (BSA) in 0.01 M phosphate-buffered saline (pH 7.4), 1% (v/v) or 5% (v/v) Tween 80 in 0.01 M phosphate-buffered saline (pH 7.4), or 5% (v/v) aqueous ethanol.

Radiolabeled and unlabeled tribufos were dissolved in acetonitrile to yield a stock solution 1280 µg/mL (4608 µg/36 mL = 128 µg/mL). One-mL aliquots were added to three containers and the acetonitrile was completely evaporated. Ten-mL portions of receptor fluid were added to each container and the contents were stirred for approximately 24 h at 32°C. A sample from each container was removed and centrifuged, and duplicate 0.25-mL portions of the supernatants were taken for radioanalysis.

Radioanalysis determined the actual mean concentration of tribufos in the selected receptor fluid [1% (v/v) Tween 80 in 0.01 M phosphate-buffered saline (pH 7.4)] was 129.0 µg/mL (100.8 ± 0.4% of the nominal concentration) following the 24-h incubation at 32°C. This was considered adequate and demonstrated that solubility was not a rate-limiting factor to absorption.

## 3. Skin preparation

**Human:** Full-thickness human skin specimens were obtained from six donors and stored at −20 ± 10°C. The skin samples were thawed at room temperature, swabbed with 70% (v/v) aqueous ethanol to remove residual fat and blood, wiped dry, and re-hydrated with distilled water.

**Rat:** Rats were euthanized by cervical dislocation. The carcasses were shaved with electric clippers and the skins removed. Connective tissue, blood, and any residual fat were removed from the dermis with absorbent tissue. Skin samples were either used on the day of euthanasia or stored at −20 ± 10°C. If frozen, the skin was thawed at room temperature and wiped with water to prepare for dermatoming.

**Rabbit:** Rabbits were euthanized by an anesthesia overdose (compound not reported). The dorsal regions of the carcasses were shaved with electric clippers and the skins removed. Connective tissue, blood, and any residual fat were removed from the dermis with absorbent tissue. Skin samples were either used on the day of euthanasia or stored at −20 ± 10°C. If frozen, the skin was treated as described for rat skin.

**Dermatome procedure:** Full-thickness skin samples were pinned to a cork board with a raised rubber surface. A mini-dermatome was used to prepare slices of skin having epidermis and some dermis (approximately 200 to 400 µm thick, measured with a digital caliper).

## 4. Study design

**Diffusion cell:** Stainless steel Scott-Dick flow-through diffusion cells were held at approximately 32°C by a water-heated manifold. Receptor fluid [1% (v/v) Tween 80 in 0.01 M phosphate-buffered saline (pH 7.4)] was pumped at a flow-rate of 1.5 mL/h

(approximately six receptor chamber content changes/h). Skin membranes were prepared from the dermatomed skin sections and placed on the receptor chamber of the cell. The donor chamber (exposure area 0.64 $cm^2$) was fixed in place, and inlet and outlet tubing connected. A diagram of the diffusion cell was included as Figure 1 on page 43 of MRID 50563601, and is included as Appendix 1 at the end of this DER.

**Membrane integrity:** Membrane integrity was determined by tritiated water ($^3H_2O$) penetration. $^3H_2O$ (250 µL) was applied to the membrane surface, the donor chamber was occluded, and distilled water was pumped through the receptor chambers at a flow rate of approximately 1.5 mL/h. Fractions were collected over 30-min intervals for a total of 3 h. At the conclusion, $^3H_2O$ remaining on the surface of the membrane was removed, the membrane surface was washed with distilled water, and the donor chamber was rinsed with distilled water to remove residual $^3H_2O$.

Radioactivity was determined in the receptor fluid samples by liquid scintillation counting (LSC). An absorption profile was constructed by plotting the amount of radioactivity absorbed per unit area skin (dpm/$cm^2$) against time (h). The absorption rate of $^3H_2O$ through each skin membrane was calculated from the slope at steady-state (dpm/$cm^2$/h) that was determined from the linear portion of the absorption profile. The permeability coefficient (Kp) for $^3H_2O$ (cm/h) was calculated as the ratio of the absorption rate and the applied concentration of radioactivity (dpm/mL). Values for Kp of $\leq 3.5 \times 10^{-3}$ cm/h for rat and human skin and $\leq 13 \times 10^{-3}$ cm/h for rabbit skin were considered acceptable. It was stated that on examination of the absorption data from skin membranes with Kp values $>3.5 \times 10^{-3}$ cm/h, if the total absorption and absorption profiles were similar to membranes with Kp values $<3.5 \times 10^{-3}$ cm/h, the data would be considered acceptable. Prior to application of the test formulations, samples of receptor fluid were collected and analyzed for background radioactivity (residual $^3H_2O$). Acceptable low levels of radioactivity were demonstrated in all of the receptor fluid samples. Additionally, the flow-rate of the receptor fluid was checked by weighing fractions collected over a measured time period (not specified) and adjusted if necessary.

**Dose determination:** Quality control portions (6.4 µL) of each dose formulation were collected throughout application. The samples were diluted to a volume of 10 mL of acetonitrile, and duplicate 0.25-mL portions were analyzed by LSC.

**Absorption and distribution:** The dose formulations (6.4 µL) were applied to the membranes with a calibrated positive displacement pipette (approximately 10 µL/$cm^2$) and were left unoccluded. The pipet tips were then placed in 10 mL acetonitrile and ultrasonicated for 30 min; duplicate 0.25-mL portions were analyzed by LSC.

Receptor fluid fractions were collected into glass vials over 2-h intervals for the duration of the experiment (10 h or 24 h).

At 10 h, or 10 h and 24 h post-application, the membranes were swabbed with 1% (v/v) aqueous Tween 80 on cotton wool buds until no radioactive residues were removed (confirmed by assessing the swabs with a radiation monitor; no further details provided). A

dry cotton wool bud was used to remove any swabbing solution remaining. The membranes were tape-stripped at termination (10 h or 24 h). The initial 1-2 tapes were considered to represent non-absorbed material (superficial stratum corneum) and were placed together in a glass vial. Subsequent tapes containing the stratum corneum were analyzed individually, and the remaining skin was retained and analyzed separately.

The receptor fluid in the receptor chamber and outlet tubing was retained and analyzed at the completion of each experiment. The diffusion cell components also were washed and the washing solutions were analyzed. All samples that were not analyzed immediately after collection were stored at approximately –20 ± 10ºC after collection.

**Quantification:** The cotton wool buds were ultrasonicated for 30 min in acetonitrile, weighed, and duplicate weighed 0.25-mL portions were radioassayed. The extraction procedures were repeated, and the second extracts were analyzed as the initial extracts. After extractions, the buds were combusted and radioassayed.

Tape strips were solubilized in a mixture of distilled water, methanol, and Triton X-405 (6:3:1 [v:v:v]) and sodium hydroxide (80 g/L) in an incubator at approximately 55°C (incubation time not provided). The solubilized mixtures were weighed, and duplicate weighed 1-mL portions were radioassayed. The skin membranes (after tape stripping) also were combined with the same solubilizing mixture and incubated at approximately 55°C (incubation time not provided). The solubilized tape-stripped skin membrane mixtures were weighed, and duplicate weighed 1-mL portions were radioassayed.

The diffusion cell components were placed in enough acetonitrile to cover and ultrasonicated for 30 min. After ultrasonication, the cell components were removed, extracts were weighed, and duplicate, weighed 1-mL portions were radioassayed.

Radioactive residues were measured by LSC. Portions of liquid samples were combined with liquid scintillant for radioactive residue determinations. Generally, radioactivity in gross amounts of less than twice background (after a 4-min count; background level was not provided) was considered below the limit of detection. Solid samples were combusted with a sample oxidizer. The efficiency of the oxidizer was determined and was >95%. Radioactive residue measurements were corrected for oxidizer efficiency.

An HPLC method with radiodetection was used for radiochemical purity checks. Details of the method were provided on pages 23-24 of MRID 50563601, and are included as Appendix 2 at the end of this DER.

5. **Statistical analysis:** Statistical analyses were not conducted; mean, standard deviation (SD), and coefficient of variance (CV) were calculated for selected data.

# II. RESULTS

## A. EPIDERMAL INTEGRITY AND SELECTION

1. **Human:** For the high-dose groups (Group 1), data were reported for all ten cells for both the 10 h and 24 h terminations. For the 10-h termination intermediate-dose group (Group 2), data were reported for nine cells. Cell #22 exceeded the acceptance criterion for membrane integrity, but the total percentage absorption and absorption profiles were in line with other cells in this group so the data were included. Cell #29 had an unacceptable recovery of radioactivity so this cell's data were not included. For the 24-h termination intermediate-dose group (Group 2), data were reported for eight cells. Cell #31 exceeded the acceptance criterion for membrane integrity, but the total percentage absorption and absorption profile were in line with other cells in this group so the data were included. Cell #35 had skin that was disrupted prior to dosing, and Cell #39 had an absorption profile that indicated disruption of the stratum corneum. The data from these two cells were not included. For the 10-h termination low-dose group (Group 3), data were reported for nine cells. Two cells (#44 and #46) exceeded the acceptance criterion for membrane integrity, but the total percentage absorption and absorption profile were in line with other cells in this group so the data were included. Cell #45 had an unacceptable recovery of radioactivity so this cell's data were not included. For the 24-h termination low-dose group (Group 3), data were reported for five cells. Three cells (#54, #55, and #56) exceeded the acceptance criterion for membrane integrity. The total percentage absorption and absorption profile for cells #54 and #56 were not in line with other cells in this group so their data were excluded. Cells #53, #55, and #57 had an unacceptable recovery of radioactivity; these cells' data were not included.

2. **Rat:** For the 10-h termination high-dose group (Group 4), data were reported for six cells with no replacements. For the 24-h termination high-dose group (Group 4), data were reported for six cells. Four cells (#R7, #R8, R10, and #R12) were found to have low recoveries of radioactivity due to an unexplained equipment malfunction, and cell R12 was noted to have disrupted skin after dosing. These cells were replaced with five other membranes. For the 10-h termination intermediate-dose group (Group 5), data were reported for five cells. Cell #R17 had an unacceptable recovery of radioactivity so this cell's data were not included and it was not replaced. For the 24-h termination intermediate-dose group (Group 5), data were reported for six cells. Three cells (#R20, R21, and #R22) were found to have low recoveries of radioactivity due to an unexplained equipment malfunction; these cells were replaced. For the 10-h termination low-dose group (Group 6), data were reported for six cells. Two cells (#R27 and #R28) exceeded the acceptance criterion for membrane integrity, but the total percentage absorption and absorption profile were in line with other cells in this group so the data were included. For the 24-h termination low-dose group (Group 6), data were reported for five cells. Cell #R31 had an unacceptable recovery of radioactivity; this cell's data were not included and it was not replaced.

3. **Rabbit:** For the 10-h termination high-dose group (Group 7), data were reported for five cells. Cell #RB37 had an unacceptable recovery of radioactivity so this cell's data were not

included and it was not replaced. For the 24-h termination high-dose group (Group 7), data were reported for five cells. Cell #RB46 was noted to have disrupted skin after dosing, but was not replaced. For the 10-h and 24-h termination intermediate-dose groups (Group 8), data were reported for six intact cells for both groups. For the 10-h termination low-dose group (Group 9), data were reported for six cells. Cell #RB65 was found to have an unacceptable recovery of radioactivity; however, it was stated that the missing radioactivity was considered to be associated with the non-absorbed portion and the data were included. For the 24-h termination low-dose group (Group 9), data were reported for six intact cells.

## B. DISTRIBUTION OF RADIOACTIVE RESIDUES

**1. Human:** Data for human skin are presented in Table 2.

### a. High dose (Group 1)

**i. 10-h termination:** Following application of 7200 µg/cm$^2$ [$^{14}$C]-tribufos (4608 µg equiv) to human skin, <0.1% of the applied dose was recovered in the receptor fluid over the 10-h exposure. The majority of the radioactivity was recovered in the skin swabs (92.6%), with 2.3% associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 0.5% of the dose. The residual skin contained 0.1%; the donor chamber accounted for 1.6%; and radioactivity recovered from the receptor chamber accounted for 0.1%. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 96.5% (4323.8 µg equiv) of the applied dose. The mean total recovery was 97.2% (4358.3 µg equiv).

The mean steady-state absorption rate at 7200 µg/cm$^2$ for 10 h could not be calculated.

**ii. 24-h termination:** Following application of 7200 µg/cm$^2$ [$^{14}$C]-tribufos (4608 µg equiv) to human skin, 0.1% of the applied dose was recovered in the receptor fluid over the 24-h period. The majority of the radioactivity was recovered in the 10-h skin swabs (91.7%), with an additional 2.1% removed with the 24-h swabs. 1.0% of the applied dose was associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 0.5% of the dose. The residual skin contained 0.2%; the donor chamber accounted for 0.7%; and radioactivity recovered from the receptor chamber accounted for <0.1%. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 95.5% (4299.5 µg equiv) of the applied dose. The mean total recovery was 96.3% (4335.1 µg equiv).

The mean steady-state absorption rate at 7200 µg/cm$^2$ for 24 h was 0.4719 µg equiv/cm$^2$/h.

### b. Intermediate dose (Group 2)

**i. 10-h termination:** Following application of 101 µg/cm$^2$ [$^{14}$C]-tribufos (64.64 µg equiv) to human skin, <0.1% of the applied dose was recovered in the receptor fluid over the 10-h exposure. The majority of the radioactivity was recovered in the skin swabs (66.2%), with

17.3% associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 7.2% of the dose. The residual skin contained 0.6%; the donor chamber accounted for 6.0%; and radioactivity recovered from the receptor chamber accounted for <0.1%. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 89.5% (61.1 µg equiv) of the applied dose. The mean total recovery was 97.4% (66.4 µg equiv).

The mean steady-state absorption rate at 101 µg/cm$^2$ for 10 h was 0.0056 µg equiv/cm$^2$/h; however, this value could only be calculated for one cell.

**ii.** **24-h termination:** Following application of 101 µg/cm$^2$ [$^{14}$C]-tribufos (64.64 µg equiv) to human skin, 0.2% of the applied dose was recovered in the receptor fluid over the 24-h period. The majority of the radioactivity was recovered in the 10-h skin swabs (60.2%), with an additional 13.3% removed with the 24-h swabs. 12.9% of the applied dose was associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 5.7% of the dose. The residual skin contained 1.7%; the donor chamber accounted for 6.6%; and no radioactivity was recovered from the receptor chamber. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 93.0% (59.4 µg equiv) of the applied dose. The mean total recovery was 100.6% (64.3 µg equiv).

The mean steady-state absorption rate at 101 µg/cm$^2$ for 24 h was 0.0089 µg equiv/cm$^2$/h.

**c. Low dose (Group 3)**

**i.** **10-h termination:** Following application of 10.1 µg/cm$^2$ [$^{14}$C]-tribufos (6.464 µg equiv) to human skin, <0.1% of the applied dose was recovered in the receptor fluid over the 10-h exposure. The majority of the radioactivity was recovered in the skin swabs (87.5%), with 8.0% associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 4.3% of the dose. The residual skin contained 0.5%; the donor chamber accounted for 0.8%; and no radioactivity was recovered from the receptor chamber. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 96.3% (5.8 µg equiv) of the applied dose. The mean total recovery was 101.1% (6.1 µg equiv).

The mean steady-state absorption rate at 10.1 µg/cm$^2$ for 10 h could not be calculated.

**ii.** **24-h termination:** Following application of 10.1 µg/cm$^2$ [$^{14}$C]-tribufos (6.464 µg equiv) to human skin, 0.1% of the applied dose was recovered in the receptor fluid over the 24-h period. The majority of the radioactivity was recovered in the 10-h skin swabs (91.6%), with an additional 2.1% removed with the 24-h swabs. 1.3% of the applied dose was associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 1.3% of the dose. The residual skin contained 0.4%; the donor chamber accounted for 1.2%; and radioactivity recovered from the receptor chamber accounted for <0.1%. The material recovered in the skin swabs, surface tape strips, and remaining on the

donor chamber was considered non-absorbed and accounted for 96.2% (5.9 µg equiv) of the applied dose. The mean total recovery was 98.1% (6.0 µg equiv).

The mean steady-state absorption rate at 10.1 µg/cm$^2$ for 24 h was 0.0053 µg equiv/cm$^2$/h.

**Table 2.** Mean (± SD) disposition of radioactive residues (mass and % applied dose) at 10 h or 24 h after topical administration of [$^{14}$C]-tribufos to excised human skin for 10 h of exposure [a]

| Termination | Concentration of tribufos | | | | | |
|---|---|---|---|---|---|---|
| | 7200 µg/cm$^2$ (4608 µg equiv) | | 101 µg/cm$^2$ (64.64 µg equiv) | | 10.1 µg/cm$^2$ (6.464 µg equiv) | |
| | µg equiv | % applied dose | µg equiv | % applied dose | µg equiv | % applied dose |
| **Receptor fluid (0-24 h)** | | | | | | |
| 10 h | 1.8 ± 2.3 | <0.1 ± 0.1 | <0.1 ± <0.1 | <0.1 ± <0.1 | <0.1 | <0.1 ± 0.1 |
| 24 h | 5.4 ± 2.8 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0.1 | <0.1 ± <0.1 | 0.1 ± 0.1 |
| **Receptor fluid (termination)** | | | | | | |
| 10 h | <4.5 | <0.1 | <0.1 ± <0.1 | <0.1 ± 0.1 | <0.1 | <0.1 |
| 24 h | <4.5 ± <4.5 | <0.1 ± <0.1 | <0.1 ± <0.1 | <0.1 ± <0.1 | <0.1 ± <0.1 | 0.1 ± 0.1 |
| **Skin** | | | | | | |
| 10 h | 4.0 ± 2.5 | 0.1 ± 0.1 | 0.4 ± 0.3 | 0.6 ± 0.4 | <0.1 | 0.5 ± 0.6 |
| 24 h | 7.7 ± 4.3 | 0.2 ± 0.1 | 1.1 ± 0.7 | 1.7 ± 1.1 | <0.1 ± <0.1 | 0.4 ± 0.3 |
| **Receptor chamber** | | | | | | |
| 10 h | 4.5 ± 12.7 | 0.1 ± 0.3 | <0.1 ± 0.1 | 0.1 ± 0.2 | ND | ND |
| 24 h | 0.5 ± 1.4 | <0.1 ± <0.1 | ND | ND | <0.1 ± <0.1 | <0.1 ± <0.1 |
| **Tape (stratum corneum)** | | | | | | |
| 10 h | 24.2 ± 10.2 | 0.5 ± 0.2 | 4.9 ± 2.2 | 7.2 ± 3.2 | 0.3 ± 0.2 | 4.3 ± 2.5 |
| 24 h | 22.1 ± 9.1 | 0.5 ± 0.2 | 3.7 ± 2.2 | 5.7 ± 3.5 | 0.1 ± <0.1 | 1.3 ± 0.5 |
| **Total absorbable** | | | | | | |
| 10 h | 34.5 ± 19.5 | 0.8 ± 0.4 | 5.4 ± 2.1 | 7.9 ± 3.1 | 0.3 ± 0.2 | 4.8 ± 2.9 |
| 24 h | 35.6 ± 12.5 | 0.8 ± 0.3 | 4.9 ± 2.9 | 7.6 ± 4.5 | 0.1 ± <0.1 | 2.0 ± 0.6 |
| **Skin swab (10 h)** | | | | | | |
| 10 h | 4151.7 ± 211.0 | 92.6 ± 4.7 | 45.2 ± 7.0 | 66.2 ± 10.3 | 5.2 ± 0.4 | 87.5 ± 6.2 |
| 24 h | 4130.6 ± 126.9 | 91.7 ± 2.8 | 38.5 ± 12.1 | 60.2 ± 19.0 | 5.6 ± 0.4 | 91.6 ± 6.3 |
| **Skin swab (24 h)** | | | | | | |
| 10 h | NA | NA | NA | NA | NA | NA |
| 24 h | 93.2 ± 74.3 | 2.1 ± 1.6 | 8.5 ± 4.0 | 13.3 ± 6.3 | 0.1 ± 0.1 | 2.1 ± 1.6 |
| **Tape (surface)** | | | | | | |
| 10 h | 102.6 ± 45.4 | 2.3 ± 1.0 | 11.8 ± 3.9 | 17.3 ± 5.7 | 0.5 ± 0.2 | 8.0 ± 3.9 |
| 24 h | 44.1 ± 24.2 | 1.0 ± 0.5 | 8.2 ± 4.7 | 12.9 ± 7.4 | 0.1 ± <0.1 | 1.3 ± 0.6 |
| **Donor chamber** | | | | | | |
| 10 h | 69.5 ± 81.0 | 1.6 ± 1.8 | 4.1 ± 1.9 | 6.0 ± 2.8 | <0.1 ± <0.1 | 0.8 ± 0.5 |
| 24 h | 31.5 ± 21.5 | 0.7 ± 0.5 | 4.2 ± 2.8 | 6.6 ± 4.3 | 0.1 ± 0.1 | 1.2 ± 0.9 |
| **Total non-absorbed** | | | | | | |
| 10 h | 4323.8 ± 135.9 | 96.5 ± 3.0 | 61.1 ± 5.3 | 89.5 ± 7.8 | 5.8 ± 0.3 | 96.3 ± 5.0 |
| 24 h | 4299.5 ± 144.8 | 95.5 ± 3.2 | 59.4 ± 3.5 | 93.0 ± 5.5 | 5.6 ± 0.4 | 96.2 ± 6.4 |
| **Total recovered** | | | | | | |
| 10 h | 4358.3 ± 127.7 | 97.2 ± 2.8 | 66.4 ± 4.3 | 97.4 ± 6.3 | 6.1 ± 0.4 | 101.1 ± 5.9 |
| 24 h | 4335.1 ± 150.5 | 96.3 ± 3.3 | 64.3 ± 3.0 | 100.6 ± 4.6 | 6.0 ± 0.4 | 98.1 ± 6.1 |

a Data were obtained from Appendix 5, Tables 1-16 on pages 116-131 of MRID 50563601; reported cells from 3-5 donors.
NA Not applicable
ND Not detected

**2. Rat:** Data for rat skin are presented in Table 3.

**a. High dose (Group 1)**

**i. 10-h termination:** Following application of 7200 µg/cm$^2$ [$^{14}$C]-tribufos (4608 µg equiv) to rat skin, 0.3% of the applied dose was recovered in the receptor fluid over the 10-h exposure. The majority of the radioactivity was recovered in the skin swabs (56.9%), with 12.2% associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 10.3% of the dose. The residual skin contained 11.3%; the donor chamber accounted for 2.6%; and radioactivity recovered from the receptor chamber accounted for 0.1%. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 71.7% (3233.7 µg equiv) of the applied dose. The mean total recovery was 93.9% (4231.5 µg equiv).

The mean steady-state absorption rate at 7200 µg/cm$^2$ for 10 h was 2.8698 µg equiv/cm$^2$/h.

**ii. 24-h termination:** Following application of 7200 µg/cm$^2$ [$^{14}$C]-tribufos (4608 µg equiv) to rat skin, 0.5% of the applied dose was recovered in the receptor fluid over the 24-h period. The majority of the radioactivity was recovered in the 10-h skin swabs (57.4%), with an additional 4.4% removed with the 24-h swabs. 9.1% of the applied dose was associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 3.1% of the dose. The residual skin contained 15.3%; the donor chamber accounted for 2.4%; and radioactivity recovered from the receptor chamber accounted for 0.4%. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 73.3% (3323.4 µg equiv) of the applied dose. The mean total recovery was 92.6% (4200.9 µg equiv).

The mean steady-state absorption rate at 7200 µg/cm$^2$ for 24 h was 2.6829 µg equiv/cm$^2$/h.

**b. Intermediate dose (Group 2)**

**i. 10-h termination:** Following application of 101 µg/cm$^2$ [$^{14}$C]-tribufos (64.64 µg equiv) to rat skin, 1.1% of the applied dose was recovered in the receptor fluid over the 10-h exposure. The majority of the radioactivity was recovered in the skin swabs (35.9%), with 19.5% associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 25.5% of the dose. The residual skin contained 10.3%; the donor chamber accounted for 1.5%; and radioactivity recovered from the receptor chamber accounted for 0.1%. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 56.9% (37.0 µg equiv) of the applied dose. The mean total recovery was 94.3% (61.3 µg equiv).

The mean steady-state absorption rate at 101 µg/cm$^2$ for 10 h was 0.2651 µg equiv/cm$^2$/h.

**ii. 24-h termination:** Following application of 101 µg/cm$^2$ [$^{14}$C]-tribufos (64.64 µg equiv) to rat skin, 3.5% of the applied dose was recovered in the receptor fluid over the 24-h period.

The majority of the radioactivity was recovered in the 10-h skin swabs (36.1%), with an additional 9.4% removed with the 24-h swabs. 17.4% of the applied dose was associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 7.4% of the dose. The residual skin contained 20.4%; the donor chamber accounted for 1.3%; and radioactivity recovered from the receptor chamber accounted for 0.2%. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 64.1% (41.2 µg equiv) of the applied dose. The mean total recovery was 95.9% (61.7 µg equiv).

The mean steady-state absorption rate at 101 µg/cm$^2$ for 24 h was 0.4456 µg equiv/cm$^2$/h.

**c. Low dose (Group 3)**

**i. 10-h termination:** Following application of 10.1 µg/cm$^2$ [$^{14}$C]-tribufos (6.464 µg equiv) to rat skin, 4.7% of the applied dose was recovered in the receptor fluid over the 10-h exposure. The majority of the radioactivity was recovered in the skin swabs (46.2%), with 38.3% associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 1.0% of the dose. The residual skin contained 10.1%; the donor chamber accounted for 1.2%; and radioactivity recovered from the receptor chamber accounted for <0.01%. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 85.6% (5.1 µg equiv) of the applied dose. The mean total recovery was 102.2% (6.0 µg equiv).

The mean steady-state absorption rate at 10.1 µg/cm$^2$ for 10 h was 0.0830 µg equiv/cm$^2$/h.

**ii. 24-h termination:** Following application of 10.1 µg/cm$^2$ [$^{14}$C]-tribufos (6.464 µg equiv) to rat skin, 8.0% of the applied dose was recovered in the receptor fluid over the 24-h period. The majority of the radioactivity was recovered in the 10-h skin swabs (44.3%), with an additional 11.7% removed with the 24-h swabs. 6.4% of the applied dose was associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 5.7% of the dose. The residual skin contained 15.0%; the donor chamber accounted for 3.7%; and radioactivity recovered from the receptor chamber accounted for 0.2%. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 66.1% (3.9 µg equiv) of the applied dose. The mean total recovery was 95.3% (5.6 µg equiv).

The mean steady-state absorption rate at 10.1 µg/cm$^2$ for 24 h was 0.0542 µg equiv/cm$^2$/h.

**Table 3.** Mean (± SD) disposition of radioactive residues (mass and % applied dose) at 10 h or 24 h after topical administration of [$^{14}$C]-tribufos to excised rat skin for 10 h of exposure [a]

| Termination | Concentration of tribufos | | | | | |
|---|---|---|---|---|---|---|
| | 7200 µg/cm$^2$ (4608 µg equiv) | | 101 µg/cm$^2$ (64.64 µg equiv) | | 10.1 µg/cm$^2$ (6.464 µg equiv) | |
| | µg equiv | % applied dose | µg equiv | % applied dose | µg equiv | % applied dose |
| **Receptor fluid (0-24 h)** | | | | | | |
| 10 h | 14.3 ± 3.4 | 0.3 ± 0.1 | 0.7 ± 0.4 | 1.1 ± 0.5 | 0.3 ± 0.2 | 4.7 ± 3.8 |
| 24 h | 22.7 ± 5.0 | 0.5 ± 0.1 | 2.3 ± 1.7 | 3.5 ± 2.6 | 0.5 ± 0.5 | 8.0 ± 8.6 |
| **Receptor fluid (termination)** | | | | | | |
| 10 h | 3.8 ± 9.2 | 0.1 ± 0.2 | 0.2 ± 0.1 | 0.3 ± 0.2 | <0.1 ± <0.1 | 0.8 ± 0.6 |
| 24 h | <4.5 | <0.1 | 0.2 ± 0.2 | 0.4 ± 0.3 | <0.1 ± <0.1 | 0.3 ± 0.2 |
| **Skin** | | | | | | |
| 10 h | 508.7 ± 258.0 | 11.3 ± 5.7 | 6.7 ± 1.3 | 10.3 ± 2.0 | 0.6 ± 0.3 | 10.1 ± 4.5 |
| 24 h | 695.4 ± 269.6 | 15.3 ± 5.9 | 13.1 ± 3.5 | 20.4 ± 5.4 | 0.9 ± 0.4 | 15.0 ± 7.3 |
| **Receptor chamber** | | | | | | |
| 10 h | 5.3 ± 6.6 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.2 | <0.1 ± <0.1 | <0.1 ± 0.1 |
| 24 h | 19.7 ± 14.2 | 0.4 ± 0.3 | 0.1 ± 0.1 | 0.2 ± 0.2 | <0.1 ± <0.1 | 0.2 ± 0.2 |
| **Tape (stratum corneum)** | | | | | | |
| 10 h | 465.8 ± 184.7 | 10.3 ± 4.1 | 16.6 ± 11.8 | 25.5 ± 18.2 | 0.1 ± <0.1 | 1.0 ± 0.4 |
| 24 h | 139.8 ± 41.7 | 3.1 ± 0.9 | 4.8 ± 7.1 | 7.4 ± 11.0 | 0.3 ± 0.2 | 5.7 ± 3.6 |
| **Total absorbable** | | | | | | |
| 10 h | 997.8 ± 350.8 | 22.1 ± 7.8 | 24.3 ± 10.9 | 37.4 ± 16.8 | 1.0 ± 0.2 | 16.6 ± 3.3 |
| 24 h | 877.5 ± 286.9 | 19.3 ± 6.3 | 20.5 ± 7.1 | 31.8 ± 11.1 | 1.7 ± 0.3 | 29.2 ± 4.3 |
| **Skin swab (10 h)** | | | | | | |
| 10 h | 2563.5 ± 636.2 | 56.9 ± 14.1 | 23.4 ± 6.8 | 35.9 ± 10.4 | 2.7 ± 0.4 | 46.2 ± 7.1 |
| 24 h | 2601.6 ± 482.1 | 57.4 ± 10.6 | 23.2 ± 3.6 | 36.1 ± 5.6 | 2.6 ± 0.2 | 44.3 ± 3.4 |
| **Skin swab (24 h)** | | | | | | |
| 10 h | NA | NA | NA | NA | NA | NA |
| 24 h | 198.8 ± 60.7 | 4.4 ± 1.3 | 6.0 ± 2.6 | 9.4 ± 4.1 | 0.7 ± 0.3 | 11.7 ± 5.0 |
| **Tape (surface)** | | | | | | |
| 10 h | 551.5 ± 269.4 | 12.2 ± 6.0 | 12.7 ± 6.4 | 19.5 ± 9.9 | 2.3 ± 0.5 | 38.3 ± 8.6 |
| 24 h | 414.2 ± 258.1 | 9.1 ± 5.7 | 11.2 ± 5.2 | 17.4 ± 8.1 | 0.4 ± 0.1 | 6.4 ± 2.3 |
| **Donor chamber** | | | | | | |
| 10 h | 118.7 ± 24.1 | 2.6 ± 0.5 | 1.0 ± 0.4 | 1.5 ± 0.6 | 0.1 ± <0.1 | 1.2 ± 0.6 |
| 24 h | 108.8 ± 58.2 | 2.4 ± 1.3 | 0.8 ± 0.4 | 1.3 ± 0.6 | 0.2 ± 0.4 | 3.7 ± 6.7 |
| **Total non-absorbed** | | | | | | |
| 10 h | 3233.7 ± 392.0 | 71.7 ± 8.7 | 37.0 ± 12.2 | 56.9 ± 18.8 | 5.1 ± 0.1 | 85.6 ± 2.4 |
| 24 h | 3323.4 ± 257.1 | 73.3 ± 5.7 | 41.2 ± 7.3 | 64.1 ± 11.4 | 3.9 ± 0.2 | 66.1 ± 4.1 |
| **Total recovered** | | | | | | |
| 10 h | 4231.5 ± 83.2 | 93.9 ± 1.8 | 61.3 ± 2.2 | 94.3 ± 3.3 | 6.0 ± 0.2 | 102.2 ± 3.7 |
| 24 h | 4200.9 ± 85.0 | 92.6 ± 1.8 | 61.7 ± 3.5 | 95.9 ± 5.4 | 5.6 ± 0.2 | 95.3 ± 2.8 |

a Data were obtained from Appendix 5, Tables 17-32 on pages 132-147 of MRID 50563601; reported cells from 2-4 donors.
NA Not applicable
ND Not detected

**3. Rabbit:** Data for rabbit skin are presented in Table 4.

**a. High dose (Group 1)**

**i. 10-h termination:** Following application of 7200 µg/cm$^2$ [$^{14}$C]-tribufos (4608 µg equiv) to rabbit skin, 0.5% of the applied dose was recovered in the receptor fluid over the 10-h exposure. The skin swabs accounted for 23.1%, with 12.4% associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 12.5% of the dose. The majority of the radioactivity was recovered in the residual skin (43.1%); the

donor chamber accounted for 0.9%; and radioactivity recovered from the receptor chamber accounted for 2.5%. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 36.4% (1578.1 µg equiv) of the applied dose. The mean total recovery was 95.0% (4118.1 µg equiv).

The mean steady-state absorption rate at 7200 µg/cm$^2$ for 10 h could not be calculated.

**ii.** **24-h termination:** Following application of 7200 µg/cm$^2$ [$^{14}$C]-tribufos (4608 µg equiv) to rabbit skin, 1.3% of the applied dose was recovered in the receptor fluid over the 24-h period. The 10-h skin swabs accounted for 16.9%, with an additional 0.9% removed with the 24-h swabs. 4.5% of the applied dose was associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 13.3% of the dose. The majority of the radioactivity was recovered in the residual skin (52.3%); the donor chamber accounted for 0.8%; and radioactivity recovered from the receptor chamber accounted for 2.8%. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 23.1% (1039.2 µg equiv) of the applied dose. The mean total recovery was 92.8% (4168.5 µg equiv).

The mean steady-state absorption rate at 7200 µg/cm$^2$ for 24 h could not be calculated.

**b. Intermediate dose (Group 2)**

**i.** **10-h termination:** Following application of 101 µg/cm$^2$ [$^{14}$C]-tribufos (64.64 µg equiv) to rabbit skin, 3.3% of the applied dose was recovered in the receptor fluid over the 10-h exposure. The majority of the radioactivity was recovered in the skin swabs (41.2%), with 7.9% associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 12.0% of the dose. The residual skin contained 28.7%; the donor chamber accounted for 1.5%; and radioactivity recovered from the receptor chamber accounted for 0.2%. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 50.6% (33.2 µg equiv) of the applied dose. The mean total recovery was 95.8% (62.7 µg equiv).

The mean steady-state absorption rate at 101 µg/cm$^2$ for 10 h was 0.5706 µg equiv/cm$^2$/h.

**ii.** **24-h termination:** Following application of 101 µg/cm$^2$ [$^{14}$C]-tribufos (64.64 µg equiv) to rabbit skin, 6.2% of the applied dose was recovered in the receptor fluid over the 24-h period. The majority of the radioactivity was recovered in the 10-h skin swabs (35.9%), with an additional 8.2% removed with the 24-h swabs. 5.0% of the applied dose was associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 6.7% of the dose. The residual skin contained 32.7%; the donor chamber accounted for 1.4%; and radioactivity recovered from the receptor chamber accounted for 0.5%. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 50.6% (33.4 µg equiv) of the applied dose. The mean total recovery was 96.9% (63.9 µg equiv).

The mean steady-state absorption rate at 101 µg/cm$^2$ for 24 h was 0.5292 µg equiv/cm$^2$/h.

**c. Low dose (Group 3)**

**i. 10-h termination:** Following application of 10.1 µg/cm$^2$ [$^{14}$C]-tribufos (6.464 µg equiv) to rabbit skin, 8.1% of the applied dose was recovered in the receptor fluid over the 10-h exposure. The majority of the radioactivity was recovered in the skin swabs (30.6%), with 21.3% associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 15.9% of the dose. The residual skin contained 18.4%; the donor chamber accounted for 1.7%; and radioactivity recovered from the receptor chamber accounted for 0.2%. The material recovered in the skin swabs, surface tape strips and remaining on the donor chamber was considered non-absorbed and accounted for 53.6% (3.2 µg equiv) of the applied dose. The mean total recovery was 97.4% (5.7 µg equiv).

The mean steady-state absorption rate at 10.1 µg/cm$^2$ for 10 h was 0.1185 µg equiv/cm$^2$/h.

**ii. 24-h termination:** Following application of 10.1 µg/cm$^2$ [$^{14}$C]-tribufos (6.464 µg equiv) to rabbit skin, 20.3% of the applied dose was recovered in the receptor fluid over the 24-h period. The majority of the radioactivity was recovered in the 10-h skin swabs (30.5%), with an additional 8.2% removed with the 24-h swabs. 10.8% of the applied dose was associated with the superficial stratum corneum (surface tape strips). The stratum corneum accounted for 5.9% of the dose. The residual skin contained 20.7%; the donor chamber accounted for 1.5%; and radioactivity recovered from the receptor chamber accounted for 0.3%. The material recovered in the skin swabs, surface tape strips, and remaining on the donor chamber was considered non-absorbed and accounted for 51.0% (3.1 µg equiv) of the applied dose. The mean total recovery was 98.6% (5.9 µg equiv).

The mean steady-state absorption rate at 10.1 µg/cm$^2$ for 24 h was 0.1308 µg equiv/cm$^2$/h.

**Table 4.** Mean (± SD) disposition of radioactive residues (mass and % applied dose) at 10 h or 24 h after topical administration of [$^{14}$C]-tribufos to excised rabbit skin for 10 h of exposure [a]

| Termination | Concentration of tribufos | | | | | |
|---|---|---|---|---|---|---|
| | 7200 µg/cm$^2$ (4608 µg equiv) | | 101 µg/cm$^2$ (64.64 µg equiv) | | 10.1 µg/cm$^2$ (6.464 µg equiv) | |
| | µg equiv | % applied dose | µg equiv | % applied dose | µg equiv | % applied dose |
| **Receptor fluid (0-24 h)** | | | | | | |
| 10 h | 23.4 ± 9.0 | 0.5 ± 0.2 | 2.1 ± 1.9 | 3.3 ± 2.9 | 0.5 ± 0.3 | 8.1 ± 5.5 |
| 24 h | 57.5 ± 17.2 | 1.3 ± 0.4 | 4.1 ± 1.2 | 6.2 ± 1.8 | 1.2 ± 0.7 | 20.3 ± 11.1 |
| **Receptor fluid (termination)** | | | | | | |
| 10 h | 0.9 ± 1.9 | <0.1 ± <0.1 | 0.7 ± 0.7 | 1.0 ± 1.0 | 0.1 ± <0.1 | 1.2 ± 0.7 |
| 24 h | <4.5 | <0.1 ± <0.1 | 0.2 ± 0.1 | 0.3 ± 0.2 | <0.1 | 0.4 ± 0.2 |
| **Skin** | | | | | | |
| 10 h | 1868.4 ± 350.2 | 43.1 ± 8.1 | 18.8 ± 3.9 | 28.7 ± 5.9 | 1.1 ± 0.2 | 18.4 ± 3.2 |
| 24 h | 2347.0 ± 309.2 | 52.3 ± 6.9 | 21.6 ± 6.3 | 32.7 ± 9.6 | 1.2 ± 0.8 | 20.7 ± 13.6 |
| **Receptor chamber** | | | | | | |
| 10 h | 106.6 ± 80.9 | 2.5 ± 1.9 | 0.1 ± 0.1 | 0.2 ± 0.2 | <0.1 | 0.2 ± 0.1 |
| 24 h | 125.7 ± 64.1 | 2.8 ± 1.4 | 0.3 ± 0.4 | 0.5 ± 0.6 | <0.1 | 0.3 ± 0.3 |
| **Tape (stratum corneum)** | | | | | | |
| 10 h | 540.8 ± 150.9 | 12.5 ± 3.5 | 7.8 ± 4.7 | 12.0 ± 7.1 | 0.9 ± 0.4 | 15.9 ± 7.3 |
| 24 h | 599.1 ± 107.4 | 13.3 ± 2.4 | 4.4 ± 3.0 | 6.7 ± 4.5 | 0.4 ± 0.3 | 5.9 ± 5.6 |
| **Total absorbable** | | | | | | |
| 10 h | 2540.0 ± 290.6 | 58.6 ± 6.7 | 29.6 ± 4.0 | 45.1 ± 6.2 | 2.6 ± 0.7 | 43.7 ± 12.2 |
| 24 h | 3129.3 ± 172.9 | 69.7 ± 3.8 | 30.6 ± 7.5 | 46.3 ± 11.3 | 2.9 ± 0.5 | 47.6 ± 9.1 |
| **Skin swab (10 h)** | | | | | | |
| 10 h | 1002 ± 162.8 | 23.1 ± 3.8 | 27.0 ± 7.5 | 41.2 ± 11.5 | 1.8 ± 0.2 | 30.6 ± 3.4 |
| 24 h | 759.9 ± 181.5 | 16.9 ± 4.0 | 23.7 ± 7.4 | 35.9 ± 11.2 | 1.8 ± 0.6 | 30.5 ± 10.8 |
| **Skin swab (24 h)** | | | | | | |
| 10 h | NA | NA | NA | NA | NA | NA |
| 24 h | 38.6 ± 27.8 | 0.9 ± 0.6 | 5.4 ± 2.0 | 8.2 ± 3.1 | 0.5 ± 0.1 | 8.2 ± 2.3 |
| **Tape (surface)** | | | | | | |
| 10 h | 538.2 ± 187.4 | 12.4 ± 4.3 | 5.2 ± 1.8 | 7.9 ± 2.7 | 1.3 ± 0.9 | 21.3 ± 14.5 |
| 24 h | 203.9 ± 92.7 | 4.5 ± 2.1 | 3.3 ± 1.7 | 5.0 ± 2.6 | 0.6 ± 0.2 | 10.8 ± 3.6 |
| **Donor chamber** | | | | | | |
| 10 h | 37.3 ± 16.4 | 0.9 ± 0.4 | 1.0 ± 0.4 | 1.5 ± 0.6 | 0.1 | 1.7 ± 0.5 |
| 24 h | 36.8 ± 16.0 | 0.8 ± 0.4 | 0.9 ± 0.6 | 1.4 ± 0.9 | 0.1 ± 0.1 | 1.5 ± 1.1 |
| **Total non-absorbed** | | | | | | |
| 10 h | 1578.1 ± 339.4 | 36.4 ± 7.8 | 33.2 ± 7.1 | 50.6 ± 10.9 | 3.2 ± 0.7 | 53.6 ± 12.3 |
| 24 h | 1039.2 ± 239.1 | 23.1 ± 5.3 | 33.4 ± 7.1 | 50.6 ± 10.7 | 3.1 ± 0.6 | 51.0 ± 10.6 |
| **Total recovered** | | | | | | |
| 10 h | 4118.1 ± 101.7 | 95.0 ± 2.3 | 62.7 ± 3.4 | 95.8 ± 5.1 | 5.7 ± 0.4 | 97.4 ± 6.1 |
| 24 h | 4168.5 ± 135.2 | 92.8 ± 3.0 | 63.9 ± 1.7 | 96.9 ± 2.6 | 5.9 ± 0.2 | 98.6 ± 2.5 |

a Data were obtained from Appendix 5, Tables 33-45 on pages 148-160 of MRID 50563601; reported cells from two donors/group, 3 donors total.
NA Not applicable
ND Not detected

**C. <u>COMPARATIVE ABSORPTION</u>:** After single applications of [$^{14}$C]-tribufos applied at 7200 µg/cm$^2$, 101 µg/cm$^2$, or 10.1 µg/cm$^2$ to human, rat, and rabbit skin membranes, total recoveries (% applied dose) of 96-101% for human skin, 93-102% for rat skin, and 93-99% for rabbit skin were obtained.

The percentage of radioactive residues (relative to the applied dose) that were removed from the site of application by the 10 h skin wash was greatest for human skin samples, followed by rat skin, then by rabbit skin (Tables 2, 3, and 4). Residues removed from human skin

were 88-93% of the applied dose for the high- and low-dose groups, with 60-66% of the applied dose removed from the intermediate-dose group. Residues removed from rat skin were 36-57% of the applied dose for all dose groups; residues removed from rabbit skin were 17-41% of the applied dose for all dose groups. For the 24-h exposure experiments, additional residues removed from human skin samples were 2.1% of the applied dose for both the high- and low-dose groups with values of 13.3% for the intermediate-dose group. Residues removed at 24 h from rat skin samples were 4-12%% of the applied dose for all dose groups; residues removed from rabbit skin were 1-8% for all dose groups.

Mean absorption profiles of [$^{14}$C]-tribufos after dermal application to human, rat, and rabbit skin are shown in Tables 5-7. For the human skin membranes, 50% of total absorption had occurred by 8 h or 12 h in the high-dose groups, by 4 h or 16 h in the intermediate-dose groups, and by 6 h and 14 h in the low dose groups after the end of the 10 and 24 h exposures, respectively. For the rat skin membranes, 50% of total absorption had occurred by 6 h or 8 h in the high-dose groups, by 8 h or 14 h in the intermediate-dose groups, and by 8 h and 16 h in the low dose groups after the end of the 10 h and 24 h exposures, respectively. For the rabbit skin membranes, 50% of total absorption had occurred by 4 h or 12 h in the high-dose groups, by 8 h or 16 h in the intermediate-dose groups, and by 8 h and 12 h in the low dose groups after the end of the 10-h and 24-h exposures, respectively. These data demonstrate that absorption of [$^{14}$C]-tribufos through human, rat, and rabbit skin continued after the 10-h skin wash. These data also provide support that radioactive residues of tribufos that remained in the stratum corneum should be considered available for absorption.

**Table 5.** Mean (± SD) absorption profiles (cumulative % of applied dose) after topical administration of [$^{14}$C]-tribufos to excised human skin after 10 h or 24 h of exposure [a]

| **Time period** | **7200 µg/cm$^2$** | | **101 µg/cm$^2$** | | **10.1 µg/cm$^2$** | |
|---|---|---|---|---|---|---|
| **(h)** | **10 h** | **24 h** | **10 h** | **24 h** | **10 h** | **24 h** |
| 0 | ND | ND | ND | ND | ND | ND |
| 2 | <0.01 | <0.01 | 0.01 ± 0.00 | <0.01 | 0.02 ± 0.01 | 0.01 ± 0.00 |
| 4 | 0.01 ± 0.00 | 0.01 ± 0.00 | **0.02 ± 0.01** | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 |
| 6 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.04 ± 0.01 | **0.03 ± 0.01** | 0.02 ± 0.01 |
| 8 | **0.03 ± 0.01** | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.04 ± 0.02 | 0.05 ± 0.03 | 0.02 ± 0.01 |
| 10 | 0.05 ± 0.02 | 0.04 ± 0.02 | 0.04 ± 0.01 | 0.05 ± 0.02 | 0.06 ± 0.03 | 0.03 ± 0.02 |
| 12 | --- | **0.06 ± 0.03** | --- | 0.08 ± 0.05 | --- | 0.05 ± 0.03 |
| 14 | --- | 0.07 ± 0.03 | --- | 0.09 ± 0.06 | --- | **0.07 ± 0.04** |
| 16 | --- | 0.08 ± 0.03 | --- | **0.10 ± 0.06** | --- | 0.08 ± 0.05 |
| 18 | --- | 0.09 ± 0.04 | --- | 0.11 ± 0.06 | --- | 0.10 = 0.06 |
| 20 | --- | 0.09 ± 0.04 | --- | 0.13 ± 0.06 | --- | 0.11 ± 0.07 |
| 22 | --- | 0.10 ± 0.04 | --- | 0.14 ± 0.07 | --- | 0.13 ± 0.08 |
| 24 | --- | 0.11 ± 0.05 | --- | 0.19 ± 0.11 | --- | 0.14 ± 0.08 |

a Data were obtained from pages 44, 46, 48, 50, 52, and 54 of MRID 50563601; standard deviation calculated by Reviewers; **bolded value** is first time period with achievement of at least 50% of overall absorption.
ND Not detected
--- No data

**Table 6.** Mean (± SD) absorption profiles (cumulative % of applied dose) after topical administration of [$^{14}$C]-tribufos to excised rat skin after 10 h or 24 h of exposure [a]

| Time period (h) | 7200 µg/cm$^2$ | | 101 µg/cm$^2$ | | 10.1 µg/cm$^2$ | |
|---|---|---|---|---|---|---|
| | **10 h** | **24 h** | **10 h** | **24 h** | **10 h** | **24 h** |
| 0 | ND | ND | ND | ND | ND | ND |
| 2 | 0.04 ± 0.03 | 0.05 ± 0.03 | 0.04 ± 0.01 | 0.06 ± 0.02 | 0.09 ± 0.03 | 0.08 ± 0.04 |
| 4 | 0.12 ± 0.06 | 0.14 ± 0.04 | 0.13 ± 0.05 | 0.14 ± 0.06 | 0.47 ± 0.39 | 0.34 ± 0.51 |
| 6 | **0.20 ± 0.08** | 0.22 ± 0.06 | 0.32 ± 0.16 | 0.33 ± 0.23 | 1.41 ± 1.29 | 0.76 ± 1.35 |
| 8 | 0.26 ± 0.09 | **0.27 ± 0.07** | **0.66 ± 0.34** | 0.70 ± 0.56 | **2.87 ± 2.56** | 1.29 ± 2.33 |
| 10 | 0.31 ± 0.09 | 0.32 ± 0.07 | 1.11 ± 0.53 | 1.21 ± 1.04 | 4.64 ± 3.74 | 1.89 ± 3.37 |
| 12 | --- | 0.36 ± 0.08 | --- | 1.49 ± 1.16 | --- | 2.82 ± 4.76 |
| 14 | --- | 0.38 ± 0.08 | --- | **1.76 ± 1.27** | --- | 3.72 ± 5.81 |
| 16 | --- | 0.40 ± 0.09 | --- | 2.08 ± 1.44 | --- | **4.63 ± 6.62** |
| 18 | --- | 0.42 ± 0.10 | --- | 2.44 ± 1.66 | --- | 5.52 ± 7.28 |
| 20 | --- | 0.44 ± 0.10 | --- | 2.82 ± 1.93 | --- | 6.35 ± 7.81 |
| 22 | --- | 0.46 ± 0.10 | --- | 3.20 ± 2.25 | --- | 7.19 ± 8.27 |
| 24 | --- | 0.48 ± 0.11 | --- | 3.52 ± 2.58 | --- | 7.98 ± 8.63 |

a Data were obtained from pages 56, 58, 60, 62, 64, and 66 of MRID 50563601; standard deviation calculated by Reviewers; **bolded value** is first time period with achievement of at least 50% of overall absorption.
ND Not detected
--- No data

**Table 7.** Mean (± SD) absorption profiles (cumulative % of applied dose) after topical administration of [$^{14}$C]-tribufos to excised rabbit skin after 10 h or 24 h of exposure [a]

| Time period (h) | 7200 µg/cm$^2$ | | 101 µg/cm$^2$ | | 10.1 µg/cm$^2$ | |
|---|---|---|---|---|---|---|
| | **10 h** | **24 h** | **10 h** | **24 h** | **10 h** | **24 h** |
| 0 | ND | ND | ND | ND | ND | ND |
| 2 | 0.15 ± 0.09 | 0.16 ± 0.03 | 0.05 ± 0.03 | 0.06 ± 0.01 | 0.12 ± 0.06 | 0.16 ± 0.15 |
| 4 | **0.31 ± 0.11** | 0.34 ± 0.04 | 0.34 ± 0.42 | 0.18 ± 0.15 | 0.99 ± 0.91 | 0.98 ± 1.28 |
| 6 | 0.39 ± 0.13 | 0.43 ± 0.04 | 1.03 ± 1.18 | 0.41 ± 0.43 | 2.82 ± 2.34 | 2.73 ± 3.01 |
| 8 | 0.47 ± 0.16 | 0.50 ± 0.07 | **2.04 ± 2.05** | 0.79 ± 0.78 | **5.41 ± 4.00** | 4.97 ± 4.69 |
| 10 | 0.54 ± 0.19 | 0.57 ± 0.10 | 3.26 ± 2.89 | 1.35 ± 1.16 | 8.10 ± 5.49 | 7.41 ± 6.12 |
| 12 | --- | **0.80 ± 0.24** | --- | 2.26 ± 1.56 | --- | **10.45 ± 7.60** |
| 14 | --- | 0.89 ± 0.29 | --- | 2.93 ± 1.61 | --- | 12.81 ± 8.56 |
| 16 | --- | 0.97 ± 0.33 | --- | **3.53 ± 1.60** | --- | 14.68 ± 9.25 |
| 18 | --- | 1.05 ± 0.36 | --- | 4.16 ± 1.55 | --- | 16.36 ± 9.80 |
| 20 | --- | 1.14 ± 0.38 | --- | 4.80 ± 1.54 | --- | 17.82 ± 10.27 |
| 22 | --- | 1.21 ± 0.39 | --- | 5.48 ± 1.60 | --- | 19.14 ± 10.70 |
| 24 | --- | 1.29 ± 0.40 | --- | 6.20 ± 1.80 | --- | 20.34 ± 11.08 |

a Data were obtained from pages 68, 70, 72, 74, 76, and 78 of MRID 50563601; standard deviation calculated by Reviewers; **bolded value** is first time period with achievement of at least 50% of overall absorption.
ND Not detected
--- No data

Total potentially absorbable tribufos was reported as the sum of radioactive residues present in the receptor fluid, the skin, the stratum corneum (tape strips 3-last), and residual residues from the receptor chamber. Radioactive materials removed by washing at 10 h or 24 h, recovered from the surface (tape strips 1-2) at the end of the exposure period, and any residual material in the donor chamber were considered unabsorbed. The percentage of the applied dose that was absorbed was lowest for human skin, increasing for rat skin, and greatest for rabbit skin. The mean percentages ranged from approximately 1% to 8% for human skin, 17% to 37% for rat skin, and 44% to 70% for rabbit skin. The mean absorption rates varied, but tended to decrease with decreasing concentration.

# III. DISCUSSION AND CONCLUSIONS

## A. INVESTIGATORS CONCLUSIONS

### Human skin

At the 10-h termination time, the material recovered in the skin swabs, surface tape strips and that remaining on the donor chamber was considered to be non-absorbed, accounting for 96.5% at the high-dose level, 89.5% at the intermediate-dose level, and 96.3% at the low-dose level.

At the 24-h termination time, the material recovered in the skin swabs, surface tape strips and that remaining on the donor chamber was considered to be non-absorbed, accounting for 95.5% at the high-dose level, 93.0% at the intermediate-dose level, and 96.2% at the low-dose level.

The absorption profiles suggest that the absorption of [$^{14}$C]-tribufos continued with time following removal of the formulation at 10 h and less than 75% of total absorption had occurred at the midpoint of the study. It is therefore considered that the material remaining in the stratum corneum should be recognised as available for absorption.

Therefore, at the 10-h termination time, the dose that was considered to be potentially absorbable (material recovered in the skin, material in the stratum corneum (tape strips 3-last one), receptor fluid and on the receptor chamber) accounted for 0.8% at the high-dose level, 7.9% at the intermediate-dose level, and 4.8% at the low-dose level. At the 24-h termination time, the dose that was considered to be potentially absorbable accounted for 0.8% at the high-dose level, 7.6% at the intermediate-dose level and 2.0% at the low-dose level.

The steady-state absorption rates for radioactivity after application of [$^{14}$C]-tribufos were low (0.0053-0.4719 µg test substance equivalents/cm$^2$/h) demonstrating that the test substance does not rapidly penetrate the skin when applied as the EC formulation concentration.

### Rat skin

At the 10-h termination time, the material recovered in the skin swabs, surface tape strips and that remaining on the donor chamber was considered to be non-absorbed, accounting for 71.7% at the high-dose level, 56.9% at the intermediate-dose level and 85.6% at the low-dose level.

At the 24-h termination time, the material recovered in the skin swabs, surface tape strips and that remaining on the donor chamber was considered to be non-absorbed, accounting for 73.3% at the high-dose level, 64.1% at the intermediate-dose level and 66.1% at the low-dose level.

The absorption profiles suggest that the absorption of [$^{14}$C]-tribufos continued with time following removal of the formulation at 10 h and less than 75% of total absorption had occurred at the midpoint of the study. It is therefore considered that the material remaining in the stratum corneum should be recognised as available for absorption.

Therefore, at the 10-h termination time, the dose that was considered to be potentially absorbable (material recovered in the skin, material in the stratum corneum (tape strips 3-last one), receptor fluid and on the receptor chamber) accounted for 22.1% at the high-dose level, 37.4% at the intermediate-dose level and 16.6% at the low-dose level. At the 24-h termination time, the dose that was considered to be potentially absorbable accounted for 19.3% at the high-dose level, 31.8% at the intermediate-dose level and 29.2% at the low-dose level.

The steady-state absorption rates for radioactivity after application of [$^{14}$C]-tribufos were low (0.0542-2.8698 µg test substance equivalents/cm$^2$/h) demonstrating that the test substance does not rapidly penetrate the skin when applied as a dilution of the EC formulation.

**Rabbit skin**

At the 10-h termination time, the material recovered in the skin swabs, surface tape strips and that remaining on the donor chamber was considered to be non-absorbed, accounting for 36.4% at the high-dose level, 50.6% at the intermediate-dose level and 53.6% at the low-dose level.

At the 24-h termination time, the material recovered in the skin swabs, surface tape strips and that remaining on the donor chamber was considered to be non-absorbed, accounting for 23.1% at the high-dose level, 50.6% at the intermediate-dose level and 51.0% at the low-dose level.

The absorption profiles suggest that the absorption of [$^{14}$C]-tribufos continued with time following removal of the formulation at 10 h and less than 75% of total absorption had occurred at the midpoint of the study. It is therefore considered that the material remaining in the stratum corneum should be recognised as available for absorption.

Therefore, at the 10-h termination time, the dose that was considered to be potentially absorbable (material recovered in the skin, material in the stratum corneum (tape strips 3-last one), receptor fluid and on the receptor chamber) accounted for 58.6% at the high-dose level, 45.1% at the intermediate-dose level and 43.7% at the low-dose level. At the 24-h termination time, the dose that was considered to be potentially absorbable accounted for accounting for 69.7% at the high-dose level, 46.3% at the intermediate-dose level and 47.6% at the low-dose level.

The steady-state absorption rates for radioactivity after application of [$^{14}$C]-tribufos were low (0.1185-0.5706 µg test substance equivalents/cm$^2$/h) demonstrating that the test

substance does not rapidly penetrate the skin when applied as a dilution of the EC formulation.

**B. REVIEWER COMMENTS:** After single applications of [$^{14}$C]-tribufos applied at 7200 µg/cm$^2$ , 101 µg/cm$^2$, or 10.1 µg/cm$^2$ to excised human, rat, and rabbit skin sections, acceptable total recoveries of ≥90% were obtained.

The percentage of radioactive residues (relative to the applied dose) that were removed from the site of application by the 10-h skin wash was greatest for human skin samples, followed by rat skin, then by rabbit skin.

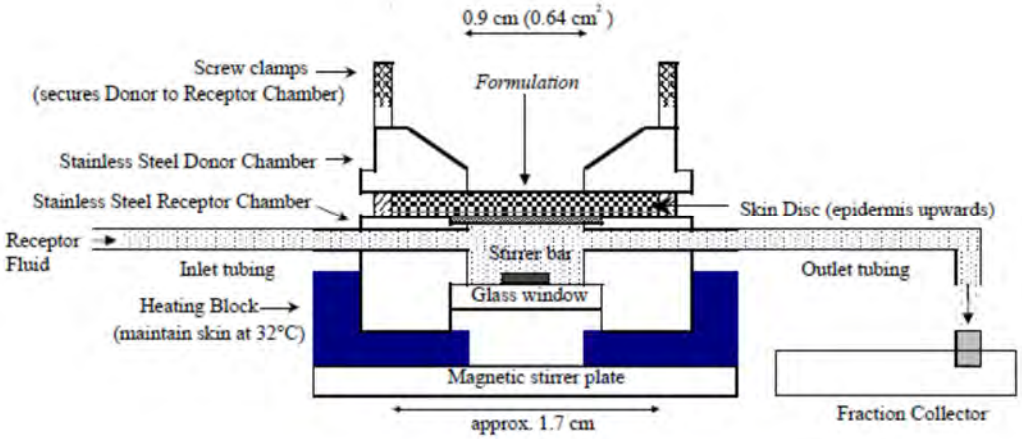
The *in vitro* dermal absorption for tribufos is ~8% in humans, ~37% in rats and ~46% in rabbits.

This study is classified **acceptable / non-guideline.** It was stated that it was performed in accordance in compliance with:

- Section 7.3 of Annex – Part A of Commission Regulation (EU) No. 545/2011 using the OECD Test Guideline 428 (April 2004) and the corresponding OECD Guidance Document for conducting *in vitro* skin absorption studies (March 2004); and
- Guidance on Dermal Absorption, EFSA Journal 2012; 10(4):2665

**C. STUDY DEFICIENCIES:** No study deficiencies were noted.

**Appendix 1.** Flow-through diffusion cell design.




*(copied from page 43 of MRID 50563601)*

**Appendix 2.** HPLC method with radiodetection.

Column: Kinetex XB-C18 (25 cm x 4.6 mm internal diameter)
Mobile phase: A: Water; B: Acetonitrile

| Gradient | Time (minutes) | %A | %B |
|---|---|---|---|
| | 0 | 25 | 75 |
| | 12 | 25 | 75 |
| | 16 | 0 | 100 |
| | 20 | 0 | 100 |
| | 25 | 25 | 75 |
| | 30 | 25 | 75 |

Flow rate: 1.0 mL/min
Column temperature: 20°C
Radioactivity detector: Flow-through system using a liquid scintillant cell
Software: Laura, version 1.4 or 4.1 (LabLogic Systems Ltd)
UV wavelength: 240 nm

*(copied from page 23-24 of MRID 50563601)*